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Original communication

What is the appropriate approach in sex determination of hyoid bones?

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ABSTRACT

The hyoid bone is characterized by sexually dimorphic features, enabling it to occasionally be used in the sex determination aspect of establishing the biological profile in skeletal remains. Based on a sample of 298 fused and non-fused hyoid bones, the present paper compares several methodological approaches to sexing human hyoid bones in order to test the legitimacy of osteometrics-based linear discriminant equations and to explore the potentials of symbolic regression and methods of geometric morphometrics. In addition, two sets of published predictive models, one of which originated in an indigenous population, were validated on the studied sample. The results showed that the hyoid shape itself is a moderate sex predictor and a combination of linear measurements is a better representation of sex-related differences. The symbolic regression was shown to exceed the predictive powers of linear discriminant function analysis when two models based on a logistic and step regression reached 96% of correctly classified cases. There was a positive correlation between discriminant scores and an individual's age as the sex assessment was highly skewed in favour of males. This suggests that the human hyoid undergoes age-related modifications which facilitates determination of male bones and complicates determination of females in older individuals. The validation of discriminant equations by Komenda and Černý (1990) and Kindschud et al. (2010) revealed that there are marked inter-population and inter-sample differences which lessened the power to correctly determine female hyoid bones.

1. Introduction

The human hyoid bone is known to bear sexually dimorphic features noticeable even from early stages of postnatal life.[1] Adult male hyoids are reported as significantly larger than those of females and this is the same case for absolute values of the widths and lengths of the greater horns and hyoid body.[2,3] In shape, female bones are typically wider; yet, the basis of the sexual dimorphism is presumed to lie in variability of the total length than in changes of width parameters.[4] Additional sex-related differences have been described in the shape of hyoid body[5] where female bones are predominantly assigned with types characterised by rounded lower borders with the lowest corners situated closely to the midsagittal plane, whereas the male type is described as either rectangular in shape or rounded with the lowest corners placed further apart. Ultimately, the role of asymmetry has been stressed as a sexually dimorphic feature.[6]

The magnitude of observed sexual dimorphism is expressed to a degree which allows hyoid bones to be used for practical purposes, i.e., as a sex predictor while assessing an individual's biological profile from skeletal remains. To date, there have been numerous studies which have produced predictive equations for sexing human hyoids.[7–9] While variable in the quality and quantity of input parameters, these studies have reported rates of accuracy ranging from 82% to 96% of correctly classified specimens. For separate anatomical elements, the rate of accuracy has been markedly lower. For instance, in the case of the hyoid body, Reesink et al.[2] reported accuracy as little as 79% for correctly classified specimens.

Like for many other skeletal elements in human body, the success of sex assessment using hyoid bones is affected by the manifestation of sexually dimorphic traits within a given population, inter-population shifts in patterns of sex-related differences as well as

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the appropriateness of a methodological approach applied to extract predictive models. The majority of predictive models have been grounded on linear distances processed by linear discriminant function analysis.[10–12] It is widely known that although linear-based statistics facilitates and speeds up computations of any kind, it may also trivialise the true biological complexity of input parameters. For predicting binary output, such as that of sex diagnosis (male/female), an alternative method of applying logistic regression has been used.[13–15] Recently, another innovation compensating for trivial input data has arisen in the form of 2D or 3D spatial data processed by methods of geometric morphometrics (GM). GM has been considerably successful in being utilized for all sorts of biological and biomedical studies, skeletal morphology included, where several papers proclaimed a better rate of accuracy if used for the purpose of sex determination.[16–21]

The present paper represents an attempt to deal with sex discrimination of the human hyoid bones by applying a variety of methodological approaches, from simple univariate discrimination to multivariate discriminant analysis to complex symbolic regression and ultimately to a model based on the approach of geometric morphometrics. In addition, the study aims to explore the reliability of two sets of published predictive models, one of them having originated in the population indigenous to the studied sample.

2. Main body

2.1. Material

The sample was composed of 298 hyoid bones, extracted at medico-legal autopsies, from individuals ranging in age from 20 to 89 years. Both fused ($N = 224$) and non-fused ($N = 74$) bones were included in the study. The distribution of sex was slightly in favour of males (153:145) (Table 1). All specimens were of Czech nationality and of European ancestry. Height and weight data were also collected. Any pathologically changed and/or damaged bones due to the cause of death or lab preparation were excluded from the sample.

2.2. Methods

Bones harvested at autopsy were manually cleaned of adherent soft tissues, plunged into alcohol solution and, once dried, placed into labelled plastic bags. For each bone, a set of 9 linear measurements as described below were taken by using Somet, a digital calliper (Fig. 1a). The osteometric parameters were selected according to their accessibility and facility on a dried bone as well as to the presumed sexual dimorphism. The lesser horns were present in 30 hyoid bones, of which 7 were present bilaterally along with 18 on the right and 19 on the left body side. Given the small sample size, measurements of lesser horns were disregarded.

Table 1
Table summarizing demographic information about the studied sample. Data are grouped in regards to sex and presence of fusion between hyoid body and greater horns. Mean values for height, weight and age in respective groups are displayed.

Sex	Fusion	*N*	%	Height	Weight	Age
Males $N = 153$	Bilateral fusion	112	37.58	174.65	84.29	62.15
	Right fusion	10	3.36	171.00	79.20	63.90
	Left fusion	11	3.69	172.18	78.27	64.09
	Non-fused	20	6.71	176.45	87.55	50.89
Females $N = 145$	Bilateral fusion	112	37.58	161.59	70.81	66.60
	Right fusion	9	3.02	163.00	80.67	70.56
	Left fusion	5	1.68	167.00	74.80	64.80
	Non-fused	19	6.38	163.74	72.89	62.79
Total		298	100.00	168.47	78.05	63.58

2.2.1. Linear measurements

THL – total hyoid length measured from the most anterior point of the hyoid body to the midpoint of the distance between tips of right and left greater horns.
THW – total hyoid width measured as the maximum distance between the lateral edges of the posterior ends of the greater horns.
BH – body height measured as the distance between the upper and lower edge of the body in the mid–sagittal plane.
BL – body length measured as the distance between the lateral edges of the hyoid body.
CHIdx, CHIsin – height of greater horn measured as the distance between the upper and lower edge at the anterior end of the greater horn (non-fused bones) or, alternatively, the distance from the upper to lower edge of the fusion line connecting the hyoid body with the right/left greater horn (fused bones).
CLdx, CLsin – length of greater horn measured as the distance from the central part of the anterior end to the distal tip (non-fused bones) or, alternatively, the distance from the central part of the fusion line to the distal tip of the greater horn (fused bones).
BT – upper anterior-posterior thickness of the body measured as the distance between the most anterior and the most posterior point of the hyoid body (as a rule located in the upper portion).

In addition to the linear measurements, the hyoid shape was described by a configuration of 23 landmarks (Fig. 1b). The set covered endpoints of the linear measurements and an additional set of 8 intermediate points (2 pairs for each side) placed along the lower and upper ridge of the greater horns. The 3D Cartesian coordinates of all 23 landmarks were recorded by MicroScribe G2LX digitizer and subsequently treated by the generalised Procrustes analysis (GPA). GPA is composed of subsequent transformations which ultimately produce a set of shape variables (Procrustes coordinates) invariant to scale, rotation and translation. In order to explore the impact of asymmetry of the sexual dimorphism variance observed, the Procrustes coordinates were divided into symmetrical and asymmetrical components. The symmetrical component included averaged right and left paired landmarks while unpaired landmarks were attributed with a zero *x*-axis. The asymmetrical component was then qualified as the difference between the original and an ideal symmetrical configuration which was included into the symmetrical component. In addition, global hyoid size was expressed in terms of the centroid size (CS), a side-product of landmark scaling in GPA.

2.3. Asymmetry

Body side variations in studied variables were tested as follows. For the linear measurements, the paired *t*-test was used, while in case of the shape variables, the asymmetry was explored by the Procrustes ANOVA of shape variables design.[22]

2.4. Sexual dimorphism

For linear measurements, the sex-related differences were summarised by the descriptive statistics and tested by univariate (*t*-test) and multivariate (Hotelling τ^2) tests. Assumptions for both were explored by the F-test (homogeneity of variance), the Shapiro–Wilk's (normality) and the Levene's tests (homogeneity of covariance matrices).

In order to predict an individual's sex, the linear measurements and shape variables (symmetrical and asymmetrical component) were processed separately by the standard linear discriminant function analysis. In the case of shape variables, differences between male and female bones were expressed in terms of the

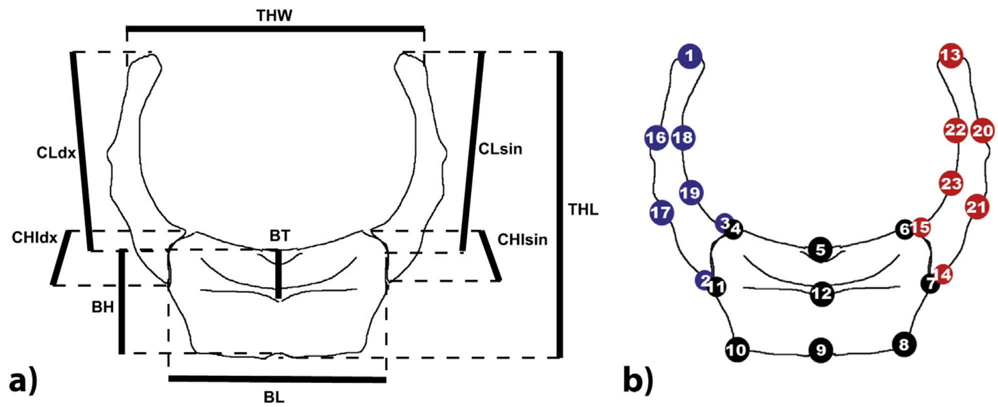

Fig. 1. Scheme illustrating a set of measurements (a) and recorded landmarks (b). Coloured circles indicate partial configurations for right and left greater horns while black filled circles refer to the configuration of landmark describing hyoid body. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Procrustes and Mahalanobis distances. The probability of both parameters was provided by the permutation test with 10 000 permutes.

In order to develop predictive models which would not assume linear relations among studied parameters, genetic modelling and symbolic regression was conducted. The symbolic regression searches for the optimal function of a given input data based on *a priori* selected sets of mathematical building-blocks (arithmetic, exponential, squashing functions) and error metric.

The general symbolic regression model had forms as follows:

$$\text{sex} = f(\text{THL}, \text{THW}, \text{BH}, \text{BL}, \text{CHI}, \text{CI}, \text{BT})$$

Of potential mathematical building-blocks, arithmetic, exponential and squashing functions were selected. Provided models were listed in an order given by values of R^2 goodness of fit. The model with the highest R^2 goodness of fit was taken as the optimal solution for the given selected criteria. The entire computation procedure was performed with the Eureqa Formulize. Eureqa Formulize is a scientific data mining software package available freely at http://www.nutonian.com/download/.

The rate of accuracy of all established predictive models was expressed as a percentage of the correctly classified specimens.

The Procrustes fit and most of the statistics were executed using MorphoJ software.[23] For statistical analyses which were not incorporated into MorphoJ software, Statistica 10 was used. For all tests, statistical significance was set at a 5% confidence level.

2.5. Validation of published predictive models on a studied sample

Although there are numerous predictive equations published for sexing the hyoid bone, the majority of their measurements are meant to be carried out with either RTG images or digital photographs. For dried bones, equations by Komenda & Černý[7] and Kindschuh et al.[9] are available. From each publication, 6 types of equations were selected – a pair incorporating the total length and width for fused bones, a pair incorporating partial dimensions measurable on non-fused bones and a pair incorporating body dimensions only (Table 2). If a bilateral measurement was required (for instance length of the greater horn) then the right and left measurements were averaged.

2.6. Measurement error

For linear measurements, intra-observer error was tested on a subsample of 40 fused and 25 non-fused hyoid bones selected randomly from the studied sample. Differences between the two datasets were tested by the paired *t*-test and the error was expressed in terms of absolute and relative technical error of measurement (TEM) and Cronbach's alpha coefficient of reliability. In addition to the total subsample, the latter was also computed for fused and non-fused bones separately. The precision of shape data acquisition was tested upon a subsample of 25 bones, selected randomly from fused bones only. Again, only intra-observer error was explored. The error was computed by the Procrustes ANOVA of shape variables design[22] and by two-factor ANOVA for size variable (centroid size) with individuals and the number of digitising sessions as factors.

The paired *t*-test revealed no significant differences for repeated linear measurements at a 5% level of accuracy. Values of Cronbach's alpha exceeding in all but one instance with a value of 0.9 suggested a high level of internal consistency in the studied dataset. The only exception was BT measurement which fell below 0.9 (0.874) in fused bones and, in terms of relative TEM, yielded an error over 8%. The absolute TEM differed in CHI and BT measurements (Mann–Whitney *U* Test, p-value < 0.05) when compared between fused and non-fused bones (Table 3).

In regards to the shape variables, the analysis revealed that the individual amount of variation exceeded the digitalisation error by a substantial amount (data not shown) and therefore should not be of any concern for the subsequently performed shape analysis.

3. Results

3.1. Descriptive statistics

The descriptive statistics (Table 4) computed for fused and non-fused hyoids suggested smaller values of linear measurements in non-fused bones. When tested for differences via Mann–Whitney *U* test, the fused and non-fused bones showed statistically significant differences in all comparable linear measurements except for BH and BT. No correlation between measurements and age was revealed if the male and female bones were pooled. Partial correlation controlling for sex, however, uncovered statistically significant correlations with age for measurements THL, BL, CHI, CL with Pearson's *R* ranging from 0.14 to 0.29. The strongest correlation was shown for CHI. For non-fused greater horns, the correlation between age and CHI was shown to be invalid.

The paired *t*-test revealed no statistically significant differences between right and left body side for either of bilateral measurements, CHI ($t = -0.201$, $p = 0.841$) and CL ($t = -1.761$, $p = 0.079$).

Table 2
An overview to published discriminant equations selected for validation on the studied sample. Note: $A = 2$ (European population).

	N	Equation	Cutoff point	Claimed rate of accuracy (M/F)
Kindschuh et al. (2010)				
For fused hyoid bones	1	$D = (0.133)(THL) + (0.219)(BL) + (0.444)(CHI) + (-0.107)(A) - 12.598$	0.0715	84.4% (87.3%/81.9%)
	2	$D = (0.153)(THL) + (0.220)(BL) + (0.395)(CHI) - 13.123$	0.0640	84.4% (87.3%/81.9%)
For non-fused hyoid bones	3	$D = 0.210(BL) + 0.416(BH)+0.498(CHI) + 0.118(A) - 13.047$	−0.13	84.3% (80.5%/89.1%)
	4	$D = 0.240(BL) + 0.413(BH)+0.430(CHI) - 13.006$	−0.13	85.2% (82.2%/89.1%)
For hyoid bodies only	5	$D = 0.311(BL) + 0.498(BH) + 0.027(A) - 12.85$	−0.092	82.8% (80.6%/85.4%)
	6	$D = 0.313(BL) + 0.495(BH) + 0.027(A) - 12.827$	−0.092	82.8% (80.6%/85.4%)
Komenda & Černý (1990)				
For fused hyoid bones	1	$D = 84.59 - 0.39(BH) - 0.99(BL) - 1.30(CHI) - 0.84(CL) - 0.51(THW)$	0	96.20%
	2	$D = 84.67 - 0.57(BH) - 1.13(BL) - 0.94(CL) - 0.52(THW)$	0	95.70%
For non-fused hyoid bones	3	$D = 63.14 - 0.56(BH) - 1.17(BL) - 0.92(CL)$	0	95.20%
	4	$D = 40.4 - 1.19(BH) - 1.01(CL)$	0	88.90%
For hyoid bodies only	5	$D = 37.48 - 0.71(BH) - 1.24(BL)$		91.90%
	6	$D = 32.69 - 1.36(BL)$	0	91.30%

These results were also valid when the analysis was processed with fused and non-fused bones separately (data not shown). Inversely, Procrustes ANOVA yielded statistically significant results for asymmetry in the overall shape as well as the body. Based on a subsample of fused bones, the mean asymmetry was manifested in unequal sloping of the greater horns and the shape of the inferior border of the body. For non-fused bones, the analysis revealed the presence of asymmetry in the body as well as the greater horns. Besides shape variables, the presence of asymmetry was equally noted in the size of the greater horns (Table 5).

3.2. Linear discriminant function analysis

For the set of fused bones, all measurements exhibited statistically significant differences between male and female bones and the same applied to the non-fused bones (Table 4). When performed on the entire set of 7 measurements, the standard discriminant analysis provided a predictive model (LDA1, Table 6) which correctly classified 92.4% of the total specimens. Male hyoids were provided with a slightly higher rate of accuracy (92.8%) in comparison to female bones (92%) (Table 8). When carried out on a subsample of non-fused bones, the discriminant analysis yielded a model (LDA2, Table 6), which predicted a correct assessment in 84.93% cases (85% in males and 84.85% in females) (Table 9).

When the 95% posterior probability rule was taken into account, then as much as 32.59% of cases (distributed approximately equally across sexes) fell into the "indeterminate" class while in cases where a decision was made, the specimens were correctly sexed in 98% of cases (97.4% in males and 98.6% in females). Once the same was carried out on the equations derived from non-fused bones, it was revealed that as much as 60.8% of cases were classified as indeterminate with a large prevalence for females (92%–8%).

Subsequently, the set of discriminant scores was correlated with body size measurements and age in order to uncover hidden relationships in sexing hyoid bones. While discriminant scores did not show any correlation with age when sex pooled ($R = 0.04$, $p > 0.05$), they did correlate with weight ($R = 0.36$, $p < 0.05$) and height ($R = 0.62$, $p < 0.05$). Separately for each sex, no correlation with size measurements was revealed. For age, however, both male and female scores showed positive dependency ($R = 0.27$ for males and $R = 0.3$ for females). This suggests that the discriminant power for recognising male bones increased with age and similarly decreased for recognising female bones. In other words, with advancing age, the hyoid bones became more masculine, i.e., larger and more robust.

The same was carried out on discriminant scores of the equation established for the non-fused hyoids (LDA 2). Similar to the previous case, the scores correlated positively with height and weight, but not with age. For males, a positive correlation was revealed for height ($R = 0.35$, p-value < 0.05) and age ($R = 0.33$, p-value < 0.05). For females, none of the explored relationships were identified as statistically significant.

Table 3
Intra-observer error quantified for linear measurements.

	Abs TEM	Rel TEM	Cronbach's alpha		
			Total	Fused	Non-fused
THL	0.30	0.88		0.992	
THW	0.52	1.22		0.994	
BH	0.09	0.89	0.997	0.997	0.990
BL	0.72	3.17	0.970	0.970	0.971
CHI	0.18	2.81	0.983	0.980	0.990
CL	0.53	1.80	0.986	0.986	0.986
BT	0.47	8.11	0.927	0.874	0.975

Table 4
Descriptive statistics and results of comparison by t-test for male and female linear measurements acquired on samples of fused and non-fused hyoid bones.

	M ($N = 112$)		F ($N = 112$)		t	p-Value
	Mean	SD	Mean	SD		
Fused bones						
THL	40.084	2.978	34.076	3.003	15.036	<0.05
THW	46.505	6.899	42.551	5.852	4.625	<0.05
BH	11.535	1.032	9.889	0.934	12.543	<0.05
BL	26.611	2.405	21.969	2.296	14.806	<0.05
CHIdx	8.090	1.034	6.537	0.880		
CHIsin	8.089	1.034	6.392	0.952		
CHI (averaged)	8.103	1.025	6.372	0.891	13.729	<0.05
CLdx	32.865	2.825	29.137	2.810		
CLsin	32.586	2.972	29.141	2.710		
CL (averaged)	32.719	2.739	29.139	2.653	8.946	<0.05
BT	6.848	1.325	5.681	0.991	7.477	<0.05
	M ($N = 41$)		F ($N = 33$)		t	p-Value
	Mean	SD	Mean	SD		
Non-fused bones						
BH	11.648	1.780	10.485	1.827	2.262	<0.05
BL	24.553	3.616	20.485	3.114	5.113	<0.05
CHIdx	7.157	0.990	5.892	0.522		
CHIsin	7.145	0.627	5.977	0.431		
CHI (averaged)	7.150	0.774	5.940	0.506	7.164	<0.05
CLdx	31.466	3.036	28.592	2.401		
CLsin	31.347	3.391	28.132	2.338		
CL (averaged)	31.432	3.085	28.486	2.363	3.937	<0.05
BT	6.572	1.168	5.494	0.991	4.199	<0.05

Table 5
Procrustes ANOVA results of asymmetry expressed in shape and size variables within fused bones and separate anatomical elements.

	Effect	SS	MS	*df*	*F*	*P* (param.)
Shape fused bones	Individual	2.309	0.00032	7040	2.77	<0.0001
	Side	0.062	0.00207	30	17.47	<0.0001
	Ind*Side	0.782	0.00012	4884	0.37	1.0000
	Residual	0.059	0.00031	80564	186	
Shape body	Individual	0.028	0.00244	11	0.53	0.8389
	Side	0.546	0.06065	9	13.27	0.0003
	Ind*Side	0.041	0.00457	9	1.33	0.2040
	Residual	4.889	0.00344	1420		
Centroid size (greater horns)	Individual	0.050	0.00071	71	2.17	0.0007
	Side	0.018	0.01824	1	55.70	<0.0001
	Ind*Side	0.023	0.00033	71	12.37	0.0776
	Residual	0.0001	0.00003	2		
Shape (greater horns)	Individual	2.600	0.00262	994	7.95	<0.0001
	Side	0.470	0.03360	14	15.26	<0.0001
	Ind*Side	2.188	0.00220	994	3.08	0.0003
	Residual	0.020	0.00071	28		

3.3. Symbolic regression

In the first round, the symbolic regression provided a logistic-regression model with a complexity of 60 items, fit of 0.102, R^2 of 0.901 and a mean square error of 0.0255. In comparison, for the same round, the most powerful linear model had the complexity of 7 items, fit of 0.458, R^2 of 0.542 and a mean square error of 0.277. Once squashing functions had been added, the second round yielded a solution of a step function model with complexity of 72 items, fit of 0.153, R^2 of 0.852 and a mean square error of 0.0382 as the best fit.

For fused bones, the applications provided two models:

$$\text{sex} = \text{logistic}(171.6{*}\text{BL} + 8.419{*}\text{THL}{*}\text{BH} + 277.5{*}\text{CHI}{*}\cos(\sin(44.66{*}\text{BL})) - 9135)$$

$$\text{sex} = \text{step}((\text{BL} + \text{CHI}{*}\text{erf}(\text{THL} - 33.87) - 30.96 - \text{erfc}(\text{BL} - 24.86){*}\tanh(6.466e26 - 5.956e25{*}\text{BH}))$$

The rates of accuracy for the models were 96.34% and 96.43% for the first and second round, respectively. Interestingly, the second equation provided a perfectly balanced rate of accuracy for males and females (Table 7).

3.4. Geometric morphometrics

Comparison of means of CS between males and females yielded statistically significant differences ($t = 15.61$, p-value < 0.05). Similarly, statistically significant differences in the global hyoid size were revealed for separate anatomical elements, body ($t = 2.624$, p-value $= 0.011$) and averaged greater horns ($t = 3.603$, p-value $= 0.001$). In all cases, male specimens were on average larger than females. Comparing t-values, the largest portion of sexual dimorphism was accounted for by the entire bone, followed by the size of greater horns. A simple univariate discrimination based on a mid-point between male and female means of the centroid size provided an accuracy of 85.3% with 83% in males and 87.5% in females.

Table 6
Predictive models and descriptive statistics of discriminant scores for sex determination on hyoid measurements established for fused (LDA1) and non-fused (LDA2) bones.

	LDA 1	LDA 2
	$D = 0.2975{*}\text{THL} + 0.0253{*}\text{THW} + 0.1535{*}\text{BH}+0.1134{*}\text{BL} + 0.3855{*}\text{CHI}-0.1827{*}\text{CI} + 0.1582{*}\text{BT}-14.6885$	$D = 0.24993{*}\text{BH} + 0.16218{*}\text{BL} + 0.61905{*}\text{CHI} + 0.08990{*}\text{CI} + 0.10756{*}\text{BT}-13.97004$
Eigenvalue	2.0819	1.09524
Males	1.42537	0.937454
Females	−1.40976	−1.136308
Total	0.01405	−0.09943

Table 7
Mean values of discriminant scores for published predictive models under validation.

		Males	Females	Total	Cut-off point
Fused bones	Kindschuh 1	1.949	−0.639	0.655	0.0715
$N_M = 114/N_F = 113$	Kindschuh 2	2.070	−0.559	0.755	0.064
	Komenda 1	−7.735	2.686	−2.524	0
	Komenda 2	−8.013	4.522	−1.746	0
Non-fused bones	Kindschuh 3	0.765	−1.139	−0.084	−0.130
$N_M = 39/N_F = 32$	Kindschuh 4	0.783	−1.162	−0.084	−0.130
	Kindschuh 5	0.640	−1.204	−0.182	−0.092
	Kindschuh 6	0.624	−1.225	−0.201	−0.092
	Komenda 3	−0.702	4.831	1.766	0
	Komenda 4	−1.235	4.635	1.383	0
	Komenda 5	−5.207	−0.848	−3.263	0
	Komenda 6	−1.026	7.094	2.595	0

The shape analysis showed that the most distinctive differences between male and female bones were located in the global shape, the shape of the body, inclination or declination of the body and sloping and vertical height of the greater horns. Female hyoids were shaped into a wide V-like appearance with a trapezoid-shaped, vertically positioned body with less steeply sloping greater horns. Male hyoids were U-shaped and featured rectangular, dorsally declined bodies and flattened, steeply sloping greater horns (Fig. 2). Discriminant analysis conducted on Procrustes coordinates provided a model with an accuracy rate reaching nearly 81% of correctly classified cases with a better classification rate for females (81.25%). Unlike for linear measurements, none of statistically significant correlations comparable to those between discriminant scores and age was revealed for shape variables.

Of the anatomical elements, a higher sex discriminant power was revealed for the averaged configurations of right and left greater horns (72%). Both Procrustes and Mahalanobis distance, however, showed no statistically significant differences either for greater horns or hyoid body (Table 10).

3.5. Validation of published predictive models

Descriptive statistics for discriminant scores suggested that in contrast to published cut-off points, the studied sample exhibited tendencies towards more masculine types of hyoid bones (Table 7). As a consequence, for both sets of discriminant equations, female bones tended to be misclassified more easily than male bones. For the fused bones, this trend was shown to be consistent in all applied equations (Table 8).

The opposite trend was observed in equations aimed at non-fused bones, i.e., the separate elements exhibited a trend toward feminine morphology, except for the Komenda 5 equation. Consequently, the misclassification rate was skewed greatly towards the male hyoid in all but one equation (Table 9).

When sex was pooled, the discriminant scores were correlated with body size parameters (weight, height) although no correlation was revealed with the individual's age. Male and female scores both correlated with age (from $R = 0.21$ to $R = 0.28$) with a subtly stronger correlation in females and no correlation with body size

Table 8
Goodness of fit statistics for newly established equations and equations under validations designed to be used for fused hyoid bones.

	LDA 1	Symbolic regression 1	Symbolic regression 2	Kindschuh 1	Kindschuh 2	Komenda 1	Komenda 2
χ^2 statistic	1.402	0.465	0.296	5.460	11.058	9.363	7.985
G^2 statistic	35.365	14.454	16.293	55.068	75.920	80.554	79.406
Males (%)	92.86	99.06	96.43	97.32	97.32	91.96	89.29
Females (%)	91.96	92.94	96.43	80.36	73.21	75.89	78.57
Total (%)	92.41	96.34	96.43	88.84	85.27	83.93	83.93

parameters hinting a trend towards masculinisation with advancing age equivalent to that seen in reference sample-based linear discriminant function analysis.

For the non-fused bones, discriminant scores correlated positively with height in all equations and with weight in all but one (Komenda 5 equation) when the dataset was sex pooled. No correlation was revealed with age. When males and females were treated separately, discriminant scores correlated with height in all but the Komenda 5 equation and with age in Komenda 3 equation in males. None of the sets of scores correlated with height, weight or age in females.

4. Discussion

Hyoid bones obtained from autopsies or burials can be useful for identifying foul play in neck injuries,[24–29] as evidence of survived ante mortem trauma[30] and they can provide information about characteristics of an individual's biological profile.[7,8]

In agreement with previous studies,[4,7–9,31] the hyoid bone manifests itself as a sexually dimorphic structure in size and shape. Male bones are generally larger than female bones. Of the linear measurements selected, the highest magnitude of sexual dimorphism is concentrated in the total anterior-posterior length of the hyoid bone, followed by the body length and height of the anterior end of greater horns. Given the lack of measurability of the total hyoid length and width in non-fused bones, the height of the anterior end is also the most sexually dimorphic dimension when separate hyoid elements were taken into account. The same measurement is also revealed as the most age-dependent when sex was controlled. This indicates that the fusion line between a greater horn and the body tends to thicken with advanced age but the trend, although observable in both males and females, differs in the timing of its onset.

Our results are generally in agreement with Pollard et al.[4] and with Mukhopadhyay[32] that female bones are typically wider in shape and that the highly variable length relative to the width causes the observed sex differences. Based on linear measurements only, the total hyoid length was indeed far more variable than the hyoid width. However, against expectations, the true sex differences were located in the hyoid body rather than in the length of greater horns. This implies that variations in the total length are not determined by the total distance from the fusion line to the distal tip of the greater horn but by a combination of all factors that determine overall hyoid body size, spatial arrangement and bending of the greater horns. The results of the univariate size analysis appear to contradict when the centroid size of the greater horns is revealed as more sexually dimorphic than the hyoid body. Again, the sex-related differences were due to variation in the vicinity of the anterior end of the greater horn than from pure differences in the total length of the greater horn.

With regards to shape, the greatest degree of sexual dimorphism was, once again, apparent when the hyoid bone was considered as whole. Interestingly, the asymmetry of the greater horns was absent in linear measurements, but it was pronounced when performed with the advanced morphometric approach. No sex differences were observed in the asymmetrical component, which contradicts Papadopoulos et al.,[6] who concludes that asymmetry in the hyoid is somehow linked to the sex of the individual.

The observed differences between fused and non-fused bones emphasise the relevance of developing different equations for both situations. In fused bones, the measurements were slightly larger if their endpoints were located on the fusion line between the body and greater horns. The distinction between the lateral edges of the body and the anterior end of the greater horns was unclear in many cases as the connective tissue formed a protruded bridge spreading over both ends. This complicated the precision in determining the endpoints. A higher rate of intraobserver error in these measurements for fused bones supports this explanation. On the other hand, the remaining measurements that did not exhibit differences of this kind also showed larger average values for the fused bones.

The accuracy and reliability of a given technique are two aspects of critical importance in sex determination. While the accuracy refers to the degree of precision within a reference sample, the reliability corresponds to how dependable a method is if applied outside a reference sample. Using 7 linear measurements, the discriminant equation for fused bones provided accuracy rate of 92% of correctly classified specimens. For non-fused bones and 5 linear measurements, the rate decreased to 84% of correctly assigned cases. Those are equivalent or even better results when compared with Kim et al.[8] who claimed a success rate of 88.2%, or Miller et al.[33] with 72.2% or Kindschud et al.[9] with 82.2%–85.2%. The best result was reported by Komenda and Černý[7] who, for geographically relevant specimens, reported an accuracy level of 88.9%–96.4%. In opposition to other skeletal elements, the shape of the hyoid bone *in sensu stricto* was identified as a moderate predictor of an individual's sex when, for the entire morphology, the discriminant function analysis performed around 80% and 72% and 67% if the landmarks of greater horns and of bodies were involved, respectively.

Table 9
Goodness of fit statistics for newly established equations and equations under validations which are designed to be used for non-fused hyoid bones.

	LDA 2	Kindschuh 3	Kindschuh 4	Kindschuh 5	Kindschuh 6	Komenda 3	Komenda 4	Komenda 5	Komenda 6
χ^2 statistic	1.61	2.10	2.99	3.63	4.020	7.973	4.455	11.62	11.13
G^2 statistic	21.53	23.98	28.78	31.34	31.655	38.82	29.98	47.53	41.19
Males (%)	85.00	82.93	82.93	78.05	75.61	65.85	73.17	95.12	63.41
Females (%)	84.85	84.85	84.85	81.82	84.85	90.91	90.91	54.55	96.97
Total (%)	84.93	83.78	83.78	79.73	79.73	77.03	81.08	77.03	78.38

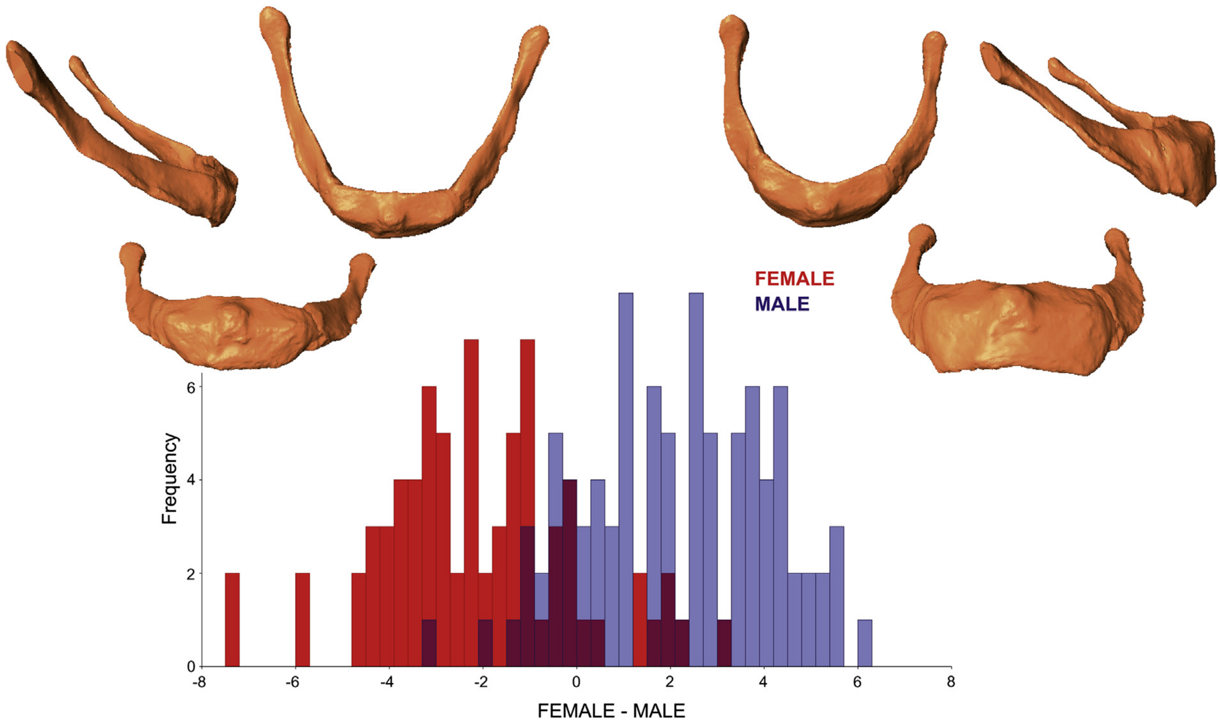

Fig. 2. Sex differences in shape of the hyoid bone. Additional 3D graphics were prepared with the help of Landmarks software. A polygonal mesh representing an actual bone was, prior to being morphed into a displayed morphology, adjusted to a starting shape by a configuration as provided by the MorphoJ software. The warped polygonal models correspond to discriminant scores of −4 and 4 for female and male bones, respectively.

An innovation of this research is incorporation of symbolic regression into the decision making. For the linear measurements in question, the best fit was identified as a logistic function and step function model. In both cases, the final levels of accuracy exceeded those of the standard linear discriminant analysis by approximately 4%. Still, searching for the most optimal function can be rather time-consuming. The analysis was left running on a standard desktop PC for a significant amount of time until the mean square error did not change for more than 60 min. For comparison, the best linear equation was achieved in less than 1 s (mean error of 0.277), the mean square error of 0.05 in approximately 3000 s (5 min) and an error less than 0.03 which included the best fit was achieved in approximately 100 000 s ($\sim$27 h). The time requirements can be easily reduced if one sets limitations to building blocks or the program searches for a specific *a priori* selected set of functions. Despite the obvious time limitations, symbolic regression may provide a new impulse into finding the most appropriate criteria for sexing human skeletal remains.

As a binding factor for linear measurement-based models, the magnitude of masculinity increases with age. For either newly established or revised equations it meant that, in advanced age, males were determined at a greater level of accuracy than females. Conversely, if the method assessed a bone as female, there was a lesser chance that the result would be incorrect. The masculinisation of the hyoid bone did not appear to be related to body size parameters. This suggests that it is erroneous to expect a more masculine hyoid bone in taller males and such individuals will not be assessed more accurately when a predictive model based on hyoid bones is applied. Having not observed an equivalent connection to discriminant scores produced on shape variables, we can assume that this trend affects size, but not shape parameters.

There is an undeniable presence of inter-population differences in the morphology of hyoid bones. Still, the reliability of two sets of currently available equations tested on the studied sample revealed better results for equations developed outside the indigenous population. In terms of the overall reliability levels, the validation for the equations for fused bones by Kindschuh et al.[9] equalled or even exceeded the reliability levels claimed by the authors. The equations by Komenda and Černý,[7] in turn, decreased below the reported levels reaching as little as 84% of correctly classified specimens instead of the reported 96% and 95% of correctly sexed specimens.

For both sets of equations, when considered independently, the percentage of correctly classified male and female bones as much as followed the same pattern – the male hyoid bones were less misclassified than female hyoid bones. This is a shortcoming shared by many other sexing techniques of the human skeleton. The phenomenon commonly known as "sexism in sexing"[34] refers to the fact that there is a directional trend in misclassification of either males or females by applied variables. For non-fused bones, the rate

Table 10
Rates of accuracy for classifying male and female hyoid bone yielded by the discriminant function analysis.

	% Correct females	% Correct males	% Correct total	Procrustes distance	*P*-values for permutation tests	Mahalanobis distance	*P*-value
Fused bones (SC)	81.25%	80.53%	80.89%	0.0411	<0.0001	1.836	<0.0001
Fused bones (AsC)	61.61%	64.60%	63.11%	0.0074	0.5520	0.7964	0.4244
Body (SC)	69.7%	65.00%	67.12%	0.0385	0.5246	0.9913	0.1978
Averaged greater horns	65.63%	77.50%	72.22%	0.0354	0.2492	1.272	0.0791

did not reach the published potentials in any of the cases. Still, it was consistent with the original pattern where female hyoids were more easily recognisable than male hyoids. The average discriminant scores, however, were heavily skewed towards males in the equation aimed for fused bones and towards females in the non-fused bone-based models. This suggests that the studied sample exhibited larger, more masculine traits when the global morphology was taken into account and *vice versa* when separate elements were concerned. Overviewing the compositions of variables within each equation and comparing average values, it can be concluded that while the total length, total width and height of the anterior end of greater horn and body height appeared to reach higher values with a smaller range of variance for the studied sample in comparison to those of Komenda and Černý[7] and Kindschuh et al.,[9] the body and greater horn lengths exhibited comparable or smaller values suggesting more female-like size of separate hyoid elements for the studied sample. The inter-sample variation is understandable. Firstly, inter-population variations are a widely acknowledged problem for sexing human bones, including hyoid bones.[35] Factors that largely interfere with the magnitude and pattern of sexual dimorphism at inter-population level, or even at inter-sample level, include a shift in time, genetic history and location. The sample by Kindschuh et al.[9] was derived from the Robert J. Terry Anatomical Collection while the sample of Komenda and Černý[7] was composed of autopsy specimens dated to the late 1980s (1986–1987). It may be short-sighted to argue exclusively with a secular trend similar to the stature or cranial height as hyoid morphology was shown to be independent of a body size parameter. Still, the marked shift towards more masculine hyoid bones merits further investigation. Alternatively, inconsistencies in measurement techniques, sample or hidden patterns in discriminant analysis could add to observed differences.

In conclusion, there are evident potentials for increasing the rates of accuracy in assessing hyoid bones. This paper has demonstrated that an implementation of advanced symbolic regression-based models can be one of the solutions for improving this accuracy.

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Conflict of interest

None declared.

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